le 4140-01-P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Institutes of Health (NIH) Office of Science Policy (OSP): Proposed Changes to the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)

AGENCY: National Institutes of Health, HHS.

ACTION: Notice.

SUMMARY: The National Institutes of Health (NIH) seeks input on a proposal to revise the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) to include specific considerations and requirements for conducting research involving gene drive modified organisms (GDMO) in contained research settings. NIH is proposing to update the NIH Guidelines to clarify minimum containment requirements, propose considerations for performing risk assessments, and define additional institutional responsibilities regarding Institutional Biosafety Committees (IBCs) and Biosafety Officers (BSOs). The proposed revisions are specific to GDMO research subject to the NIH Guidelines, conducted in contained settings and are consistent with the recommendations of the NIH Novel and Exceptional Technology Research Advisory Committee report, Gene Drives in Biomedical Research (NExTRAC Report). NIH does not currently support research involving potential field release of GDMOs and the NIH Guidelines pertain to contained research; accordingly, no changes regarding potential field release are being proposed in this Notice. NIH is also proposing revisions to the NIH Guidelines to harmonize with the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition regarding the Risk Group (RG) categorization of West Nile Virus (WNV) and Saint Louis Encephalitis Virus (SLEV).

DATES: To ensure consideration, comments must be submitted in writing by [INSERT DATE 60 DAYS AFTER DATE OF PUBLICATION IN THE **FEDERAL REGISTER**].

ADDRESSES: Comments may be submitted electronically to https://osp.od.nih.gov/proposed-amendments-to-the-nih-guidelines-for-research-involving-recombinant-or-synthetic-nucleic-acid-molecules-nih-guidelines/. Comments are voluntary and may be submitted anonymously. You may also voluntarily include your name and contact information with your response. Other than your name and contact information, please do not include in the response any personally identifiable information or any information that you do not wish to make public. Proprietary, classified, confidential, or sensitive information should not be included in your response. After the Office of Science Policy (OSP) has finished reviewing the responses, the responses may be posted to the OSP website without redaction.

FOR FURTHER INFORMATION CONTACT: Caroline Young, ScM, Acting Director of the Division of Biosafety, Biosecurity, and Emerging Biotechnology Policy, Office of Science Policy, at (301) 496-9838 or SciencePolicy@od.nih.gov.

SUPPLEMENTARY INFORMATION: The NIH currently supports basic gene drive research in contained laboratory settings as the technology holds great promise for advancing public health, particularly through the potential to reduce transmission of vector-borne human diseases such as malaria, dengue, or Zika. Under certain conditions, gene drive technology enables researchers to promote the spread of certain genetic traits that has the potential to mitigate disease by driving traits through a specific species population at a faster rate with fewer reproductive cycles.

Gene drive technology presents opportunities for many life sciences applications with potential benefits to public health, agriculture, and the environment but also raise biosafety, ethical, and social concerns. To help consider issues associated with

conducting research involving GDMOs safely and responsibly, the NIH charged an advisory committee to the NIH Director, the Novel and Exceptional Technology and Research Advisory Committee (NExTRAC), to consider whether existing biosafety guidance is adequate for contained laboratory research utilizing GDMOs. The NExTRAC made multiple recommendations for strengthening NIH's existing policies and guidance, which were shared for public input and ultimately accepted by the NIH Director. These proposed changes only address the NExTRAC's recommendations pertaining to contained research. NIH does not currently support research involving potential field release of GDMOs and the NIH Guidelines pertain to contained research; as such, no changes are being proposed in this notice regarding field release research of GDMOs. NIH is seeking input on its proposal to amend the NIH Guidelines to ensure the continued responsible research involving GDMOs in contained research settings. Specifically, NIH proposes to:

1) clarify minimum containment requirements for research involving GDMOs;

2) propose considerations for risk assessment;

3) define additional institutional responsibilities for Institutional Biosafety Committees (IBCs) and Biosafety Officers (BSOs).

In addition to the amendments proposed related to contained research involving GDMOs, the NIH is seeking input on its proposal to:

1. replace the term "helper viruses" with the broader term "helper systems"; and

2. reclassify WNV and SLEV as risk group 2 agents for consistency with containment guidance provided in the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition.

Current Language and Proposed Amendments to the NIH Guidelines

A definition for gene drive is proposed to be added to Section I-E, specifically:

Section I-E. General Definitions

Section I-E-7. "Gene drive" is defined as a technology whereby a particular heritable element biases inheritance in its favor, resulting in the heritable element becoming more prevalent than predicted by Mendelian laws of inheritance in a population over successive generations.

Section II-A-3, which provides guidance for conducting a comprehensive risk assessment, has been updated in the past to provide additional guidance regarding issues that should be considered for research involving emerging technologies (e.g., guidance for research with organisms involving synthetic nucleic acids when the parent organism is not obvious). Robust risk assessment for research with GDMOs may present challenges due to different or increased risks associated with the potential to persist and spread in the environment. To address some of these challenges, Section II-A-3 is proposed to be amended to include considerations for risk assessment.

Section II-A-3 currently states:

Section II-A-3. Comprehensive Risk Assessment

In deciding on the appropriate containment for an experiment, the first step is to assess the risk of the agent itself. Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, classifies agents into Risk Groups based on an assessment of their ability to cause disease in humans and the available treatments for such disease. Once the Risk Group of the agent is identified, this should be followed by a thorough consideration of how the agent is to be manipulated. Factors to be considered in determining the level of containment include agent factors such as: virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher

containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain (see Section V-B, Footnotes and References of Sections I-IV).

While the starting point for the risk assessment is based on the identification of the Risk Group of the parent agent, as technology moves forward, it may be possible to develop an organism containing genetic sequences from multiple sources such that the parent agent may not be obvious. In such cases, the risk assessment should include at least two levels of analysis. The first involves a consideration of the Risk Groups of the source(s) of the sequences and the second involves an assessment of the functions that may be encoded by these sequences (e.g., virulence or transmissibility). It may be prudent to first consider the highest Risk Group classification of all agents that are the source of sequences included in the construct. Other factors to be considered include the percentage of the genome contributed by each parent agent and the predicted function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as they did in the original host context.

The Principal Investigator and Institutional Biosafety Committee must also be cognizant that the combination of certain sequences in a new biological context may result in an organism whose risk profile could be higher than that of the contributing organisms or sequences. The synergistic function of these sequences may be one of the key attributes to consider in deciding whether a higher containment level is warranted, at least until further assessments can be carried out. A new biosafety risk may occur with an organism formed through combination of sequences from a number of organisms or due to the synergistic effect of combining transgenes that results in a new phenotype.

A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment (see Section II-B, Containment).

The appropriate containment level may be equivalent to the Risk Group classification of the agent, or it may be raised or lowered as a result of the above considerations. The Institutional Biosafety Committee must approve the risk assessment and the biosafety containment level for recombinant or synthetic nucleic acid experiments described in Sections III-A, Experiments that Require NIH Director Approval and Institutional Biosafety Committee Approval, Before Initiation; III-B, Experiments that Require NIH OSP and Institutional Biosafety Committee Approval Before Initiation; III-C, Experiments Involving Human Gene Transfer that Require Institutional Biosafety Committee Approval Prior to Initiation; III-D, Experiments that Require Institutional Biosafety Committee Approval Before Initiation.

Careful consideration should be given to the types of manipulation planned for some higher Risk Group agents. For example, the RG2 dengue viruses may be cultured under the Biosafety Level (BL) 2 containment (see Section II-B); however, when such agents are used for animal inoculation or transmission studies, a higher containment level is recommended. Similarly, RG3 agents such as Venezuelan equine encephalomyelitis and yellow fever viruses should be handled at a higher containment level for animal inoculation and transmission experiments.

Individuals working with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or other bloodborne pathogens should consult the applicable Occupational Safety and Health Administration (OSHA) (https://www.osha.gov/) (regulation, 29 CFR 1910.1030, and OSHA publication 3127 (1996 revised). BL2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV- or HBV-infected or inoculated laboratory animals. Activities such as the production of research-laboratory scale quantities of HIV or other bloodborne pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, are performed in a BL2 facility using

the additional practices and containment equipment recommended for BL3. Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a BL3 facility, or BL3 Large Scale if appropriate, using BL3 practices and containment equipment.

Exotic plant pathogens and animal pathogens of domestic livestock and poultry are restricted and may require special laboratory design, operation and containment features not addressed in Biosafety in Microbiological and Biomedical Laboratories (see Section V-C, Footnotes and References of Sections I through IV). For information regarding the importation, possession, or use of these agents see Sections V-G and V-H, Footnotes and References of Sections I through IV.

Risk mitigation strategies employed in contained settings are not likely to differ for GDMOs compared to other gene modified organisms in the laboratory. However, given the relative newness of GDMO technology and its use in biomedical research, any risk assessment is likely to have greater uncertainty regarding potential risks. Section II-A-3 is proposed to be amended to provide additional guidance for conducting these assessments by insertion of new paragraphs five and six:

Section II-A-3 is proposed to be amended to:

In deciding on the appropriate containment for an experiment, the first step is to assess the risk of the agent itself. Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, classifies agents into Risk Groups based on an assessment of their ability to cause disease in humans and the available treatments for such disease. Once the Risk Group of the agent is identified, this should be followed by a thorough consideration of how the agent is to be manipulated. Factors to be considered in determining the level of containment include agent factors such as: virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity,

physiological activity, and allergenicity. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain (see Section V-B, Footnotes and References of Sections I-IV).

While the starting point for the risk assessment is based on the identification of the Risk Group of the parent agent, as technology moves forward, it may be possible to develop an organism containing genetic sequences from multiple sources such that the parent agent may not be obvious. In such cases, the risk assessment should include at least two levels of analysis. The first involves a consideration of the Risk Groups of the source(s) of the sequences and the second involves an assessment of the functions that may be encoded by these sequences (e.g., virulence or transmissibility). It may be prudent to first consider the highest Risk Group classification of all agents that are the source of sequences included in the construct. Other factors to be considered include the percentage of the genome contributed by each parent agent and the predicted function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as they did in the original host context.

The Principal Investigator and Institutional Biosafety Committee must also be cognizant that the combination of certain sequences in a new biological context may result in an organism whose risk profile could be higher than that of the contributing organisms or sequences. The synergistic function of these sequences may be one of the key attributes to consider in deciding whether a higher containment level is warranted, at least until further assessments can be carried out. A new biosafety risk may occur with an organism formed through combination of sequences from a number of organisms or due to the synergistic effect of combining transgenes that results in a new phenotype.

A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment (see Section II-B, Containment). The appropriate containment level may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations. The Institutional Biosafety Committee must approve the risk assessment and the biosafety containment level for recombinant or synthetic nucleic acid experiments described in Sections III-A, Experiments that Require NIH Director Approval and Institutional Biosafety Committee Approval, Before Initiation; III-B, Experiments that Require NIH OSP and Institutional Biosafety Committee Approval Before Initiation; III-C, Experiments Involving Human Gene Transfer that Require Institutional Biosafety Committee Approval Prior to Initiation; III-D, Experiments that Require Institutional Biosafety Committee Approval Before Initiation.

Research involving gene drive modified organisms may require risk assessments that incorporate a broader scope of considerations because of greater uncertainty of the technology and potential uncertainty of the impact of the newly modified organism. Specific attention must be paid to risks of an unintended release from the laboratory and the potential impact on humans, other populations of organisms, and the environment. Considerations for conducting risk assessments for research involving gene drive modified organisms might include:

1. The specific types of manipulations based on:
    a. Function or intended function of the genetic/gene drive construct (i.e., a designed or engineered assembly of sequences);
    b. Source of the genetic material (e.g., sequences of transgenes) in the construct;
    c. The modifications to the construct;

d. Whether it is possible to predict the consequences of a construct, including the recognition of an unintended gene drive (i.e., construct not specifically designed as a gene drive but nonetheless having properties of a gene drive) and the possible consequences of escape into the environment;
e. The potential ability of the gene drive to spread or persist in local populations;

2. Options for approaches to risk mitigation for specific types of risks in experiments or when dealing with a high degree of uncertainty about risks;
3. Considerations for implementing more stringent containment measures until biosafety data are accrued to support lowering containment.

Careful consideration should be given to the types of manipulation planned for some higher Risk Group agents. For example, the RG2 dengue viruses may be cultured under the Biosafety Level (BL) 2 containment (see Section II-B); however, when such agents are used for animal inoculation or transmission studies, a higher containment level is recommended. Similarly, RG3 agents such as Venezuelan equine encephalomyelitis and yellow fever viruses should be handled at a higher containment level for animal inoculation and transmission experiments.

Individuals working with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or other bloodborne pathogens should consult the applicable Occupational Safety and Health Administration (OSHA) regulation, 29 CFR 1910.1030, and OSHA publication 3127 (1996 revised). BL2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV- or HBV-infected or inoculated laboratory animals. Activities such as the production of research-laboratory scale quantities of HIV or other bloodborne pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or

aerosols, are performed in a BL2 facility using the additional practices and containment equipment recommended for BL3. Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a BL3 facility, or BL3 Large Scale if appropriate, using BL3 practices and containment equipment.

Exotic plant pathogens and animal pathogens of domestic livestock and poultry are restricted and may require special laboratory design, operation and containment features not addressed in Biosafety in Microbiological and Biomedical Laboratories (see Section V-C, Footnotes and References of Sections I through IV). For information regarding the importation, possession, or use of these agents see Sections V-G and V-H, Footnotes and References of Sections I through IV.

In 2012 when the NIH Guidelines were updated to expand the scope to cover synthetic nucleic acid molecules, Section III-C and Section III-F-1 were amended to exempt research with certain oligonucleotides based on the lower risk posed by their transient nature. These sections also outlined criteria for higher risk nucleic acids that would not be exempt (e.g., nucleic acids that replicated, were transcribed, translated, or integrated etc.). At that time, much research with oligonucleotides was likely to involve a delivery method using a recombinant nucleic acid molecule (e.g., viral vector or plasmid), and thus would still be subject to the NIH Guidelines. Since then, gene editing using CRISPR/Cas systems and non-recombinant delivery methods (e.g., lipid nanoparticles) has come into more common use. Currently, transgenic organisms with the same genetic modification may or may not be subject to the NIH Guidelines depending on the method of generation (e.g., recombinant viral vector delivery and expression of Cas9 and guide RNAs vs. lipid nanoparticle delivery of protein Cas9 and guide RNAs). Because of the higher risks associated with stable genetic modifications to viruses, cells, or organisms, Sections III-C and III-F-1 each have a criterion that precludes the exemption of nucleic acids that integrate, the main method to introduce such changes in 2012. To avoid

exempting certain gene editing approaches or GDMOs, the language in Sections III-C and III-F-1 is proposed to be amended to replace the criterion involving integration with a broader criterion covering the introduction of a stable genetic modification.

Section III-C-1 currently states in part:

Section III-C-1. Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants

Human gene transfer is the deliberate transfer into human research participants of either:

1. Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or
2. Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:
    a. Contain more than 100 nucleotides; or
    b. Possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or
    c. Have the potential to replicate in a cell; or
    d. Can be translated or transcribed.

This portion of Section III-C-1 1 is proposed to be amended to:

Section III-C-1. Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants

Human gene transfer is the deliberate transfer into human research participants of either:

1. Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or

2. Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:

a. Contain more than 100 nucleotides; or

b. Possess biological properties that enable introduction of stable genetic modifications into the genome (e.g., cis elements involved in integration, gene editing); or

c. Have the potential to replicate in a cell; or

d. Can be translated or transcribed.

Section III-F-1 currently states:

Section III-F-1. Those synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C, it is not exempt under this Section.

Section III-F-1 is proposed to be amended to:

Section III-F-1. Those synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to introduce a stable genetic modification, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C, it is not exempt under this Section.

To provide guidance on physical containment for research involving GDMOs, Section III-D is proposed to be amended in multiple subsections to require that experiments involving GDMOs be conducted at a minimum of BL2 containment to provide the

appropriate laboratory practices, containment equipment, and special laboratory design to protect laboratory workers, the public, and local ecosystems. A section specific to experiments involving GDMOs is proposed to be added as Section III-D-8. Sections III-D-4, III-D-5, and III-E-3, which cover experiments with whole animals, plants, and transgenic rodents, are also proposed to be amended to reference Section III-D-8.

Section III-D-4, which is part of Section III-D, Experiments that Require Institutional Biosafety Committee Approval Before Initiation, currently states:

Section III-D-4. Experiments Involving Whole Animals

This section covers experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms tested on whole animals. For the latter, other than viruses which are only vertically transmitted, the experiments may *not* be conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required.

Caution - Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

Section III-D-4-a. Recombinant or synthetic nucleic acid molecules, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study (see Section V-B, Footnotes and References of Sections I-IV). Animals that contain sequences from

viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III-D-4-b, Experiments Involving Whole Animals. For experiments involving recombinant or synthetic nucleic acid molecule-modified Risk Groups 2, 3, 4, or restricted organisms, see Sections V-A, V-G, and V-L, Footnotes and References of Sections I-IV. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, Footnotes and References of Sections I-IV).

Section III-D-4-b. For experiments involving recombinant or synthetic nucleic acid molecules, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Section III-D-1, Experiments Using Human or Animal Pathogens (Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems), or Section III-D-4-a, the appropriate containment shall be determined by the Institutional Biosafety Committee.

Section III-D-4-c. Exceptions under Section III-D-4, Experiments Involving Whole Animals

Section III-D-4-c-(1). Experiments involving the generation of transgenic rodents that require BL1 containment are described under Section III-E-3, Experiments Involving Transgenic Rodents.

Section III-D-4-c-(2). The purchase or transfer of transgenic rodents is exempt from the NIH Guidelines under Section III-F, Exempt Experiments (see Appendix C-VII, The Purchase or Transfer of Transgenic Rodents).

Section III-D-4 is proposed to be amended to state:

Section III-D-4. Experiments Involving Whole Animals

This section covers experiments involving deliberate transfer of recombinant or synthetic nucleic acid molecules, DNA or RNA derived from recombinant or synthetic nucleic acid molecules, or recombinant or synthetic nucleic acid molecule-modified microorganisms into whole animals and experiments involving whole animals in which the animal's genome has been altered by recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic animals). Experiments involving gene drive modified animals or experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms, except for viruses that are only vertically transmitted, may *not* be conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required (see Section III-D-8).

Caution - Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms (e.g., a gene drive; refer to Section III-D-8) or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

Section III-D-4-a. Recombinant or synthetic nucleic acid molecules, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study (see Section V-B, Footnotes and References of Sections I-IV). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under

conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III-D-4-b, Experiments Involving Whole Animals. For experiments involving recombinant or synthetic nucleic acid molecule-modified Risk Groups 2, 3, 4, or restricted organisms, see Sections V-A, V-G, and V-L, Footnotes and References of Sections I-IV. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, Footnotes and References of Sections I-IV).

Section III-D-4-b. For experiments involving recombinant or synthetic nucleic acid molecules, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Section III-D-1, Experiments Using Human or Animal Pathogens (Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems), or Section III-D-4-a, the appropriate containment shall be determined by the Institutional Biosafety Committee. Experiments involving gene drive modified animals generated by recombinant or synthetic nucleic acid molecules shall be conducted at a minimum of BL2 or BL2-N (see Section III-D-8).

Section III-D-4-c. Exceptions under Section III-D-4, Experiments Involving Whole Animals

Section III-D-4-c-(1). Experiments involving the generation of transgenic rodents that require BL1 containment are described under Section III-E-3, Experiments Involving Transgenic Rodents.

Section III-D-4-c-(2). The purchase or transfer of BL1 transgenic rodents is exempt from the NIH Guidelines under Section III-F, Exempt Experiments (see Appendix C-VII, The Purchase or Transfer of Transgenic Rodents).

Section III-D-4-c-(3). Experiments involving the generation or use of gene drive modified animals require a minimum of BL2 containment and are covered under III-D-8, Experiments Involving Gene Drive Modified Organisms.

Section III-D-5 currently states in part:

Section III-D-5. Experiments Involving Whole Plants

Experiments to genetically engineer plants by recombinant or synthetic nucleic acid molecule methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant or synthetic nucleic acid molecules, may be conducted under the containment conditions described in Sections III-D-5-a through III-D-5-e. If experiments involving whole plants are not described in Section III-D-5 and do not fall under Sections III-A, III-B, III-D or III-F, they are included in Section III-E.

This portion of Section III-D-5 is proposed to be amended to:

Section III-D-5. Experiments Involving Whole Plants

Experiments to genetically engineer plants by recombinant or synthetic nucleic acid molecule methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant or synthetic nucleic acid molecules, may be conducted under the containment conditions described in Sections III-D-5-a through III-D-5-e. If experiments involving whole plants are not described in Section III-D-5 and do not fall under Sections III-A, III-B, III-D or III-F, they are included in Section III-E. Experiments involving the generation or use of gene drive modified organisms require a minimum of BL2 containment and are described under Section III-D-8, Experiments Involving Gene Drive Modified Organisms.

Section III-D-8 is proposed to be added to state:

Section III-D-8. Experiments Involving Gene Drive Modified Organisms

Experiments involving gene drive modified organisms generated by recombinant or synthetic nucleic acid molecules shall be conducted at a minimum of Biosafety Level (BL) 2, BL2-N (Animals) or BL2-P (plant) containment.

Only transgenic rodents that may be contained under BL1 are covered under Section III-E-3. Section III-E-3 is proposed to be amended to reference the new Section III-D-8 to reinforce that research with GDMOs shall be conducted at a minimum of BL2.

Section III-E-3, which is part of Section III-E, Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation, states in part:

Section III-E-3. Experiments Involving Transgenic Rodents

This section covers experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under Section III-D-4, Experiments Involving Whole Animals.

This portion of Section III-E-3 is proposed to be amended to:

Section III-E-3. Experiments Involving Transgenic Rodents

This section covers experiments involving the generation or use of rodents in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under Section III-D-4, Experiments Involving Whole Animals or Section III-D-8, Experiments Involving Gene Drive Modified Organisms.

In the NExTRAC report, the committee recommended that NIH should require appropriate expertise in the review of gene drive research by IBC members and BSO.

Portions of Section IV-B are proposed to be amended regarding institutional responsibilities for the establishment of IBCs and requirements for BSOs.

Section IV-B-1-c currently states:

Section IV-B-1-c. Appoint a Biological Safety Officer (who is also a member of the Institutional Biosafety Committee) if the institution: (i) conducts recombinant or synthetic nucleic acid molecule research at Biosafety Level (BL) 3 or BL4, or (ii) engages in large-scale (greater than 10 liters) research. The Biological Safety Officer carries out the duties specified in Section IV-B-3.

Section IV-B-1-c is proposed to be amended to:

Section IV-B-1-c. Appoint a Biological Safety Officer (who is also a member of the Institutional Biosafety Committee) if the institution: (i) conducts recombinant or synthetic nucleic acid molecule research at Biosafety Level (BL) 3 or BL4, (ii) engages in large-scale (greater than 10 liters) research or (iii) conducts research involving gene drive modified organisms. The Biological Safety Officer carries out the duties specified in Section IV-B-3.

Section IV-B-2-a, Membership and Procedures of IBCs currently states in part:

Section IV-B-2-a-(1). The Institutional Biosafety Committee must comprise no fewer than five members so selected that they collectively have experience and expertise in recombinant or synthetic nucleic acid molecule technology and the capability to assess the safety of recombinant or synthetic nucleic acid molecule research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the

community). The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix L, Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Plants, require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix M, Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Animals, require Institutional Biosafety Committee prior approval. When the institution conducts recombinant or synthetic nucleic acid molecule research at BL3, BL4, or Large Scale (greater than 10 liters), a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see Section IV-B-3, Biological Safety Officer). When the institution participates in or sponsors recombinant or synthetic nucleic acid molecule research involving human research participants, the institution must ensure that the Institutional Biosafety Committee has adequate expertise and training (using ad hoc consultants as deemed necessary). Institutional Biosafety Committee approval must be obtained from the clinical trial site.

Section IV-B-2-a-(1) is proposed to be amended to read:

Section IV-B-2-a-(1). The Institutional Biosafety Committee must comprise no fewer than five members so selected that they collectively have experience and expertise in recombinant or synthetic nucleic acid molecule technology and the capability to assess the safety of recombinant or synthetic nucleic acid molecule research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or

environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community). The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix L, Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Plants, require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix M, Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Animals, require Institutional Biosafety Committee prior approval. When the institution conducts research involving gene drive modified organisms the institution must ensure that the Institutional Biosafety Committee has adequate expertise (e.g., specific species containment, ecological or environmental risk assessment) using ad hoc consultants if necessary. When the institution conducts recombinant or synthetic nucleic acid molecule research at BL3, BL4, or Large Scale (greater than 10 liters) or research involving gene drive modified organisms, a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see Section IV-B-3, Biological Safety Officer). When the institution conducts research with gene drive modified organisms, the impact on ecosystems should be assessed by the Institutional Biosafety Committee (see Section V-N, Footnotes and References of Sections I-IV). When the institution participates in or sponsors recombinant or synthetic nucleic acid molecule research involving human research participants, the institution must ensure that the Institutional Biosafety Committee has adequate expertise and training (using ad hoc consultants if necessary). Institutional Biosafety Committee approval must be obtained from the clinical trial site. Section IV-B-3, Biological Safety Officer (BSO), states in part:

Section IV-B-3-a. The institution shall appoint a Biological Safety Officer if it engages in large-scale research or production activities involving viable organisms containing recombinant or synthetic nucleic acid molecules.

Section IV-B-3-a is proposed to be amended to clarify the requirement for a BSO to be a member of the IBC. A new Section IV-B-3-c is proposed to be added to require a BSO for research involving GDMOs. The current IV-B-3-c sections will be re-lettered to IV-B-3-d.

Section IV-B-3-a. The institution shall appoint a Biological Safety Officer if it engages in large-scale research or production activities involving viable organisms containing recombinant or synthetic nucleic acid molecules. The Biological Safety Officer shall be a member of the Institutional Biosafety Committee.

Section IV-B-3-c. The institution shall appoint a Biological Safety Officer if it engages in recombinant or synthetic nucleic acid molecule research that involves gene drive modified organisms. The Biological Safety Officer shall be a member of the Institutional Biosafety Committee.

To emphasize that GDMOs may have an impact on ecosystems, a new footnote and reference for Sections I through IV is proposed to be added.

Section V-N is proposed to state:

Section V-N Determination of whether a gene drive modified organism has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of gene drive technology, the environment, and ecosystems in the geographic area of the research.

Since research with GDMOs shall be conducted at a minimum of Biosafety Level 2, research involving host vector system organisms modified by a gene drive will not be exempt. Therefore, the exceptions (Appendices C-III-A and C-IV-A) to Appendices C-III

and C-IV, Saccharomyces and Kluyveromyces Host-Vector Systems, respectively, are proposed to be amended.

Appendices C-III-A Exceptions and C-IV-A Exceptions currently state:

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-B which require NIH OSP and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, and Sections V-G and V-L, Footnotes and References of Sections I through IV) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates).

Appendices C-III-A Exceptions and C-IV-A Exceptions are proposed to be amended to state:

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-B, which require NIH OSP and Institutional Biosafety Committee approval before initiation; (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, and Sections V-G and V-L, Footnotes and References of Sections I through IV) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval; (iii) large-scale experiments (e.g., more than 10 liters of culture), (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, Containment Conditions

for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates), and (v) experiments involving gene drive modified organisms (Section III-D-8).

To provide additional guidance on containment for work with arthropods, Appendices G, L, and M are proposed to reference the Arthropod Containment Guidelines, which specifically outline practices and procedures for arthropod research, and the addendum Arthropod Containment Guidelines, which articulates containment practices for gene drive modified arthropods. Appendix G-III and Footnotes and References of Appendix G will also be modified to reference the current edition of the reference source BMBL and to correct an erroneous second citation of the BMBL.

Appendix G-III-A currently states:

Appendix G-III-A. Biosafety in Microbiological and Biomedical Laboratories, 5th edition, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and National Institutes of Health, Bethesda, Maryland.

Appendix G-III-A is proposed to be amended to state:

Appendix G-III-A. Biosafety in Microbiological and Biomedical Laboratories, 6th edition, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and National Institutes of Health, Bethesda, Maryland.

Appendix G-III-B currently states:

Appendix G-III-B. Biosafety in Microbiological and Biomedical Laboratories, 3rd edition, May 1993, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and NIH, Bethesda, Maryland.

Appendix G-III-B is proposed to be amended to state:

Appendix G-III-B. Arthropod Containment Guidelines, Version 3.2, 2019, and Addendum 1 Containment Practices for Arthropods Modified with Engineered

Transgenes Capable of Gene Drive, 2022, American Committee of Medical Entomology, American Society of Tropical Medicine and Hygiene, Arlington, Virginia.

Appendices L and M specify containment conditions and practices for plants and animals, respectively, that preclude the use of containment as specified in Appendix G. Both Appendices L and M will be modified to incorporate the Arthropod Containment Guidelines and cross-reference to Appendix G-III-B.

Appendix L-III-C currently states:

Appendix L-III-C. Biological Containment Practices (Macroorganisms)

Appendix L-III-C-1. Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.

Appendix L-III-C is proposed to be amended to:

Appendix L-III-C. Biological Containment Practices (Macroorganisms)

Appendix L-III-C-1. Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water. Containment for arthropods is described in the Arthropod Containment Guidelines and Addendum 1

Containment Practices for Arthropods Modified with Engineered Transgenes Capable of Gene Drive (see Appendix G-III-B).

Appendix M-III-D currently states:

Appendix M-III-D. Other research with non-laboratory animals, which may not appropriately be conducted under conditions described in Appendix M, may be conducted safely by applying practices routinely used for controlled culture of these biota. In aquatic systems, for example, BL1 equivalent conditions could be met by utilizing growth tanks that provide adequate physical means to avoid the escape of the aquatic species, its gametes, and introduced exogenous genetic material. A mechanism shall be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank, aquarium, etc.) Acceptable barriers include appropriate filtration, irradiation, heat treatment, chemical treatment, etc. Moreover, the top of the rearing container shall be covered to avoid escape of the organism and its gametes. In the event of tank rupture, leakage, or overflow, the construction of the room containing these tanks should prevent the organisms and gametes from entering the building's drains before the organism and its gametes have been inactivated.

Other types of non-laboratory animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1-P through BL4-P containment practices and procedures as specified in Appendices G and L.

Appendix M-III-D is proposed to be amended to:

Appendix M-III-D. Research with animals, which may not appropriately be conducted under conditions described in Appendix M, may be conducted safely by applying practices routinely used for controlled culture of these biota. In aquatic systems, for example, BL1 equivalent conditions could be met by utilizing growth tanks that provide

adequate physical means to avoid the escape of the aquatic species, its gametes, and introduced exogenous genetic material. A mechanism shall be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank, aquarium, etc.) Acceptable barriers include appropriate filtration, irradiation, heat treatment, chemical treatment, etc. Moreover, the top of the rearing container shall be covered to avoid escape of the organism and its gametes. In the event of tank rupture, leakage, or overflow, the construction of the room containing these tanks should prevent the organisms and gametes from entering the building's drains before the organism and its gametes have been inactivated.

Other types of animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1-P through BL4-P containment practices and procedures as specified in Appendices G and L. Containment for arthropods is described in the Arthropod Containment Guidelines and Addendum 1 Containment Practices for Arthropods Modified with Engineered Transgenes Capable of Gene Drive (see Appendix G-III-B).

The term “helper virus” is used in multiple sections of the NIH Guidelines to refer to the missing functions provided to a defective virus. However, helper systems (e.g., transient transfection systems, packaging cell lines, replicon systems, etc.) are more commonly used than a helper virus. NIH OSP has interpreted the term “helper virus” to extend to the use of helper systems because they are also associated with the risk of generation of replication competent virus. To clarify the language in the NIH Guidelines, the term “helper virus” will be replaced in Sections III-D-3, and III-E-1 with the term “helper systems”.

The risk group classification in Appendix B of two viruses, West Nile virus and St. Louis encephalitis virus, are proposed to be changed from RG3 to RG2 to be consistent with the risk assessment that is articulated in the current edition of the BMBL.

Appendix B-III-D currently states in part:

Appendix B-III-D. Risk Group 3 (RG3) - Viruses and Prions

Alphaviruses (Togaviruses) - Group A Arboviruses currently states in part:

--St. Louis encephalitis virus

Flaviviruses - Group B Arboviruses currently states in part:

--West Nile virus (WNV)

Appendix B-II-D is proposed to be amended to state:

Appendix B-II-D. Risk Group 2 (RG2) – Viruses

Alphaviruses (Togaviruses) - Group A Arboviruses

--St. Louis encephalitis virus

Flaviviruses - Group B Arboviruses

--West Nile virus (WNV)

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